DEVELOPMENT OF THE CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF PALONOSETRON IN PALONOSETRON HYDROCHLORIDE INJECTION

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ABSTRACT
This work is concerned with application of simple, precise, accurate and reproducible high performance liquid chromatographic (HPLC) method for assay of Palonosetron in Palonosetron injection on RP Kromasil C8,(100 * 4.6mm), 5µ Make Akzonobel Column using Water : Acetonitrile : Trifluoro acetic acid (700:300:0.64) as mobile phase at a flow rate of 1.2 ml/min, the detection wavelength was 210nm and the instrument was Aligent 1100/1200 series with UV/DAD detector and isocratic/gradient pump. The retention time for Palonosetron Hydrochloride was found to be 3.6 min. The proposed method is suitable for assay of Palonosetron in Palonosetron injection. Keywords: Palonosetron Hydrochloride injection, wavelength, High Performance Liquid Chromatography.

INTRODUCTION
Palonosetron Hydrochloride is an Antiemetic; selective inhibitor of type 3 serotonergic (5-HT$_3$) receptors. It is used in prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of moderately emetogenic cancer chemotherapy also in prevention of acute nausea and vomiting associated with initial and repeat courses of highly emetogenic cancer chemotherapy. Palonosetron is administered intravenously, as a single dose, 30 minutes before chemotherapy, or as a single oral capsule one hour before chemotherapy. The oral formulation was approved on August 22, 2008 for prevention of acute CINV alone, as a large clinical trial did not show oral administration to be as effective as intravenous use against delayed CINV.[1-4]
High Performance Liquid Chromatography (HPLC) It is also called as liquid chromatography (LC). It is one of the most widely used analytical techniques today, among the different chromatographic procedure due to the significant evolution in LC instrument, providing superior qualitative and quantitative results. HPLC is the fastest growing analytical technique for the analysis of drugs. Its simplicity, high speed and wide range of sensitivity make it ideal for analysis of many drugs in dosage forms as well as in biological fluids. HPLC is a separation technique where separation is accomplished by partitioning between a mobile phase (solvent) and a Stationary phase (column). HPLC differ from other types of liquid chromatography with regards to packing material of small, uniform particles utilized. The small size of particles give high column efficiencies that also results in high pressure drop across the columns, and therefore higher pressures are utilized to achieve desired flow rates. Hence it is also called as “high pressure liquid chromatography”.

Analytical methods are required to characterize drug substances and drug products composition during all phases of pharmaceutical development. Development of methods to achieve the final goal of ensuring the quality of drug substances and drug products must be implemented in conjunction with an understanding of the chemical behavior and physicochemical properties of the drug substance. This determination requires highly sophisticated methods and instruments like HPLC.

**MATERIALS AND METHOD**

**Instrument:** HPLC: Agilent 1100/1200 series with UV/DAD detector and isocratic/gradient pump.

**Chemicals**

<table>
<thead>
<tr>
<th>Material</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified water (HPLC grade)</td>
<td>Nanopure Diamond water Purification System</td>
</tr>
<tr>
<td>Trifluoroacetic acid</td>
<td>AR</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>HPLC</td>
</tr>
</tbody>
</table>
**Standard Preparation**: Transfer an accurately weighed quantity about 10.0 mg of Palonosetron HCl working standard in to 20 mL volumetric flask. Add about 10 mL of diluent to dissolve and dilute to volume with diluent; mix well. Dilute 5.0 mL of this solution to 100 mL with diluent and mix well.

**Assay Preparation**: Transfer an accurately weighed quantity of about 5000 mg of sample solution in to 10 mL volumetric flask and dilute to volume with diluent; mix evenly.

**Weight per mL determination**: Take an empty pycnometer and record the weight. Adjust the temperature of sample to about 20°C and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25°C, remove any access of the sample and weigh. Subtract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the weight per milliliter by dividing weight by volume capacity of the pycnometer.

**System Suitability**: Equilibrate the column with prescribed conditions until a stable baseline is achieved. Separately inject six replicate injections of standard preparation in to liquid chromatograph and record the chromatogram.

**Procedure**: Separately inject duplicate injections of assay preparation in to liquid chromatograph and record the chromatogram. Calculate the assay of Palonosetron in % of label claim from the Palonosetron peak area of standard preparation, assay preparation and % potency of working standard used.

- **Column**: Kromasil C8 (100 * 4.6 mm), 5 µ Make: Akzo Nobel or equivalent
- **Flow Rate**: 1.2 mL/min
- **Wavelength**: 210 nm
- **Injection Volume**: 10 µL
- **Column Temperature**: 25°C
- **Retention Time**: About 3.6 min for Palonosetron
- **Run Time**: 5 min
- **Mobile Phase**: Water : Acetonitrile : Trifluoroacetic acid (700:300:0.64)
- **Diluent**: Mobile Phase
Calculation:
\[(A_u/A_s) \times (W_1/20) \times (5/100) \times (10/W_2) \times (W_3/L.C.) \times (296.37/332.87) \times (P/100) \times (100)\]

Where,
- \(A_u\) = Mean peak area due to Palonosetron obtained with assay preparation.
- \(A_s\) = Mean peak area due to Palonosetron obtained with standard preparation.
- \(W_1\) = Weight of Palonosetron HCl working standard taken in mg.
- \(W_2\) = weight of sample taken in mg.
- \(W_3\) = Weight per mL of sample in mg/mL.
- \(L.C.\) = Label Claim of Palonosetron in mg/mL.
- \(P\) = Potency of Palonosetron HCl working standard in % on as is basis.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drug Palonosetron in Palonosetron Hydrochloride injection by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The objective of this study was to develop a rapid and sensitive HPLC method for the analysis of palonosetron in Palonosetron Hydrochloride injection using Agilent 1100/1200 series with UV/DAD detector and isocratic/gradient pump and Kromasil C8 (100 * 4.6 mm), 5 µ Make: Akzo Nobel column. The run time was set at 5 min and Palonosetron appeared on the chromatogram. When the sample was injected 6 times, the retention time of the drug was same i.e. 3.6 min. The average of 6 such determinations of peak areas are shown in Table: 1 The percentage assay value was found to be 98.3 %.
Table 1

<table>
<thead>
<tr>
<th>System Suitability Parameters</th>
<th>Results</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative standard deviation of replicate injections for Palonosetron peak area</td>
<td>0.04</td>
<td>NA</td>
</tr>
<tr>
<td>Average tailing factor for Palonosetron peak</td>
<td>1.12</td>
<td>NA</td>
</tr>
<tr>
<td>Average theoretical plates (by tangent method) for Palonosetron peak</td>
<td>6137</td>
<td>NA</td>
</tr>
<tr>
<td>Average area of replicate injections for Palonosetron peak</td>
<td>1142.09842</td>
<td>NA</td>
</tr>
<tr>
<td>Percentage assay value</td>
<td>98.3</td>
<td>NA</td>
</tr>
</tbody>
</table>

Figure 1
Diluent

Figure 2
Standard Preparation
CONCLUSION

we have developed simple, sensitive, precise and accurate HPLC method for the assay of Palonosetron. The results expressed in Table 1 for HPLC are promising. In addition to positive requirements for analytical methods, the striking advantage of all the presently developed methods is that they are economical. This methods can be employed for routine analysis of Palonosetron in Palonosetron injection.

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REFERENCES


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